

Applicant : M. Amin Arnal
 Serial No. : 09/805,354
 Filed : March 13, 2001
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Attorney's Docket No.: 00786-536001

Replace the paragraph beginning at page 7, line 11, with the following rewritten paragraph:

B1
 Figure 5 depicts an alignment of the A domains of nine alpha integrin α subunit (CD11b (SEQ ID NO:1), CD11c (SEQ ID NO:2), CD11d (SEQ ID NO:3), CD11a (SEQ ID NO:4), alpha 1 (SEQ ID NO:5), alpha 2 (SEQ ID NO:6), alpha 10 (SEQ ID NO:7), alpha 11 (SEQ ID NO:8), and alpha E (SEQ ID NO:9)). In this alignment, the invariant Ile (I316) is indicated by an arrow.

Replace the paragraph beginning at page 7, line 17, with the following rewritten paragraph:

B2
 Figure 7 is an alignment of the A-like domains of eight integrin β subunits β 3 (SEQ ID NO:10), β 5 (SEQ ID NO:11), β 6 (SEQ ID NO:12), β 1 (SEQ ID NO:13), β 2 (SEQ ID NO:14), β 7 (SEQ ID NO:15), β 8 (SEQ ID NO:16), and β 9 (SEQ ID NO:17). In this alignment, the residue corresponding to the invariant Ile in β subunits is indicated by an arrow.

Replace the paragraph beginning at page 8, line 3, with the following rewritten paragraph:

B3
 The variant polypeptides were created using standard recombinant techniques. Restriction and modification enzymes were purchased from New England Biolabs, Inc. (Beverly, MA), Boehringer Mannheim (Germany), or GIBCO BRL (Gaithersburg, MD). Site-directed mutagenesis was carried out in pGEX-4T-1 vector as described (Rieu et al. 1996 *J Biol Chem* 271:15858). The following mutagenic primers were used. IFAdel Fwd: 5'-TATAGGATCCGAGGCCCTCCGAGGGAGTCCTCAAGAGGATAG-3' (SEQ ID NO:18); Reverse: 5'-CTACTCGAGTTACTTCTCCCGAAGCTGGTTCTGAATGGTC-3' (SEQ ID NO:19); I-G reverse: 5'-CTACTCGAGTTAACCCTCGATCGCAAAGCCCTTCTC-3' (SEQ ID NO:20). Introduction of the respective mutation was confirmed by direct DNA sequencing. The PvuI-BspEI-restricted cDNA fragment of the A-domain containing the mutation was subcloned into the PvuI-BspEI-restricted CD11b cDNA, cloned into pcDNA3 plasmid, which containing full-length human CD11b (Rieu et al. 1996 *J Biol Chem* 271:15858). 11b A¹²³⁻³²¹ and 11bA¹²³⁻³¹⁵ and 11bA^{1→G} A-domains were expressed as GST fusion proteins in *Escherichia coli*

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(Michishita et al. 1993 Cell 72:857), cleaved with thrombin and purified as described Li et al. 1999 *J. Cell Biol* 143:1523. C¹²⁹ was replaced by S in all the expressed GST-A-domain fusion form to prevent formation of disulfide-linked dimmers in solution after thrombin cleavage (not shown). Purity was confirmed by SDS-PAGE analysis.
